Allelopathic effects by *Empetrum hermaphroditum* on development and nitrogen uptake by roots and mycorrhizae of *Pinus silvestris*

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Fennoscandian plant communities dominated by the evergreen dwarf shrub *Empetrum hermaphroditum* Hagerup appear to have negative effects on forest regeneration. Effects of water extracts of leaves of *E. hermaphroditum* on the development and nitrogen uptake by roots and mycorrhizae (*Paxillus involutus* Batsch (Fr.)) of Scots pine (*Pinus silvestris* L.) were studied in a greenhouse experiment. Among seedlings that did not receive *E. hermaphroditum* extract, mycorrhizal plants grew better than nonmycorrhizal plants and also had a higher shoot to root ratio. Nitrogen uptake per unit root length was 3 times faster in mycorrhizal plants. The uptake of nitrogen was 92 ng · day^{-1} per dichotomous tip as compared with 22 ng · day^{-1} per nondichotomous root tip (mycorrhizal plants had both dichotomous and nondichotomous tips, whereas uninoculated plants almost exclusively had nondichotomous tips). A low concentration of extract impaired nitrogen uptake in both mycorrhizal and nonmycorrhizal plants. Uptake per unit root length was reduced to one-third and one-tenth in mycorrhizal and nonmycorrhizal plants, respectively. Calculated per dichotomous tip per day, the uptake of nitrogen was reduced to only 0.9 ng. Additions of extract also retarded increases in root length and the numbers of root tips, although less markedly, but the percentage of dichotomous tips was more strongly reduced. *Empetrum hermaphroditum* extract also strongly inhibited the growth of *Paxillus involutus* on modified Melin–Norkrans agar medium. The spread of mycorrhizal infection (as indicated by the percentage of dichotomous tips) and uptake of nutrients by roots and mycorrhizae were more sensitive to the extract than seed germination and radicle growth, which were demonstrated to be affected under field conditions.

Key words: allelopathy, *Empetrum hermaphroditum*, forestry, mycorrhiza, nitrogen.

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Les communautés végétales fennoscandiennes (fennoscandian) dominées par l'arbuste nain pérenne, *Empetrum hermaphroditum* Hagerup, semble avoir des effets négatifs sur la regénération forestière. Les auteurs ont étudié, en serres, les effets d'extraits aqueux de feuilles de l'*E. hermaphroditum* sur le développement et l'absorption de l'azote par les racines et les mycorhizes (*Paxillus involutus* Batsch (Fr.)) du pin sylvestre (*Pinus silvestris* L.). Chez les plants qui n'ont pas reçu d'extrait de l'*E. hermaphroditum*, ceux qui portaient des mycorhizes avaient une meilleure croissance que ceux qui n'en avaient pas; ils avaient également un rapport tige à racine plus élevé. L'absorption de l'azote par unité de longeur de racine, chez les plants mycorhizés, était 3 fois plus rapide que chez les plants sans mycorhize. L'absorption de l'azote est de 92 ng · jour^{-1} par racine dichotomique, comparativement à 22 ng · jour^{-1} par racine non-dichotomique (les plantes mycorhiziennes avaient des racines dichotomiques aussi bien que non-dichotomiques alors que les plantes non-inoculées avaient presque seulement des racines non-dichotomiques). Une faible concentration d'extrait ralentit l'absorption de l'azote chez les plants mycorhizés aussi bien que non-mycorhizés. L'absorption par unité de longeur de racine est réduite à un tier et un dixième chez les plantes mycorhizées et non-mycorhizées, respectivement. Calculée sur la base de dichotomie par jour, l'absorption de l'azote est réduite à 0.9 ng. Des additions d'extrait retardent également mais plus faiblement, les augmentations des longeurs des racines et les nombres d'apex racinaires, mais le pourcentage d'extrémités dichotomiques est plus fortement réduit. L'extrait de l'*E. hermaphroditum*, aussi, inhibe fortement la croissance du *Paxillus involutus* sur milieu modifié de Melin–Norkrans gélosé. L'extension de la colonisation mycorhizienne (tel qu'indiqué par le pourcentage d'extrémités dichotomiques) et l'absorption des nutrients par les racines et les mycorhizes, sont plus sensibles à l'extrait que la germination des graines et la croissance des radicules, dont l'inhibition a été démontré aux champs.

Mots clés : allélopathie, *Empetrum hermaphroditum*, foresterie, mycorhize, azote.

[Traduit par la rédaction]

Introduction

Insufficient natural regeneration and suppressed seedling growth of conifers were ascribed to plant communities dominated by the evergreen dwarf shrub *Empetrum hermaphroditum* Hagerup in northern Scandinavia for many years (Sarvas 1950; Ebeling 1979; Hagner 1984). Recently, *E. hermaphroditum* was identified as a source of allelopathic activity (Zackrisson and Nilsson 1992; Nilsson and Zackrisson 1992). Reduced seed germination, stunted seedling growth, and high mortality of Scots pine (*Pinus silvestris* L.) were shown in bioassays and field experiments to be due to water soluble inhibitors produced in leaf glands of *E. hermaphroditum* (Zackrisson and Nilsson

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1992). Analysis of water extracts from *E. hermaphroditum* leaves suggested that 3-methoxy-5,3′-dihydroxy-dihydrostilbene or batatasin III may be one of the compounds responsible for inhibition of seed germination of aspen (*Populus tremula* L.) (Odén et al. 1992). These observations prompted more detailed studies on the effects of *E. hermaphroditum* on Scots pine roots and its possible influence on mycorrhizal symbioses.

Mycorrhizae are widely recognized to improve seedling survival in natural environments (Harley and Smith 1983). Leachate from leaves of vascular plants (Olsen et al. 1971; Ponder 1986; Coté and Thibault 1988), lichens (Brown and Mikola, 1974; Fisher 1979; Goldner et al. 1986; Perry and Choquette 1987), and exudates from roots (Robinson 1972) can inhibit the growth of ectomycorrhizal fungi. Organic toxic substances originating in forest floor litter may also adversely affect mycorrhizal formation (Handley 1963; Schoenberger and Perry 1982; Rose et al. 1983). In contrast, other studies demonstrated stimulation of mycorrhizal fungi by water extracts from plant leaves and litter (Rose et al. 1983) and also demonstrated that mycorrhizal seedlings exposed to phytotoxins grew better than nonmycorrhizal seedlings (Hanson and Dixon 1987; Coté and Thibault 1988). Most studies on allelopathic interference by plants or litter either focused on the effects on mycorrhizal colonization of tree roots in the field or were pure culture tests using various ectomycorrhizal fungi.

Observations concerning the effect of allelochemicals on the function of mycorrhizal symbioses are scarce. Fisher (1979) speculated that the nutrient uptake by mycorrhizal symbionts of conifers might be impaired by *Cladonia* lichens, but as he pointed out, no distinction could be made between translocation from root to shoot and uptake of ^{32}P in his experiment. Brown and Mikola (1974) also made an attempt to assess this by applying ^{32}P to the growth medium of mycorrhizal and nonmycorrhizal Scots pine seedlings. Their results are difficult to interpret because uptake was not related to the biomass or length of the root systems.

Quantitative studies are needed to critically assess the potential importance of allelopathy on functional aspects of roots and mycorrhizae, e.g., nutrient uptake. The purposes of this study were to determine whether *E. hermaphroditum* leaf extracts were inhibitory to (*i*) the development of roots and spread of mycorrhizal infection by the ectomycorrhizal fungus *Paxillus involutus* Batsch (Fr.) on the root system and (*ii*) the nitrogen absorption by roots and mycorrhizae. The aim was thus to identify the target of action rather than to establish if there is allelopathic interference in the field. However, a standardized experimental protocol was used to allow comparisons with field studies (Nilsson and Zackrisson 1992; Zackrisson and Nilsson 1992).

Materials and methods

Plant material, preparation of water extracts, and growth conditions

Green leaves of *E. hermaphroditum* were collected during November 1989 at Rovågern, northern Sweden (63°50′N, 20°30′E), air dried at room temperature, and stored at −18°C before use. A 5% stock solution of water extract was prepared by soaking 50 g of dry green leaves in 1 L of distilled water while slowly stirring for 48 h. The extract was filtered through Munktell No. 3 filter paper to remove debris. The solution had an osmotic potential of 43 mmol · kg^{-1} and a pH of 4.2. It was stored at −18°C.

Scots pine seeds (full sib, Swedish (BD2054 × BD2075, 1976)) were surface sterilized (30% H_2O_2, 5 min) and germinated at 20°C on moist, oven-sterilized (130°C, 12 h) quartz sand (diameter 0.85–0.95 mm) in the greenhouse. Seedlings emerged after 8–11 days. After 24 days, the seedlings were transferred to sterilized 0.7-L plastic pots containing 1000 g of the oven-sterilized sand with distilled water added to field capacity (Table 1). Half of the batch of seedlings were inoculated with mycelium of the ectomycorrhizal fungus *Paxillus involutus* (isolate No. 87017) cultivated on modified Melin–Norkrans agar medium (MMN) (Marx 1969). Pots were placed in the greenhouse under 20-h illumination and temperatures of 17–25°C on polyethylene nets, approximately 1 cm above the experimental table to reduce the risk of fungal contamination of uninoculated seedlings.

TABLE 1. Description of the experiment

Day	Event	Days after inoculation	Days after start of extract treatments	Cumulative no. of extract applications
63	Start of extract treatments	39		
84	Harvest 1	60	21	3
105	Harvest 2	81	42	6
136	Harvest 3	112	73	11
168	Harvest 4	144	105	13

NOTE: At day 24, seedlings were inoculated with the fungus; at day 33, the nutrient regime was started; at day 63, the extract treatments were started. Each harvest was 80 seedlings.

The pots were watered three times a week, initially with 150 mL/pot of tap water to support seedling acclimation. A complete nutrient solution with macronutrients in the optimal proportions recommended by Ingestad (1979) was then given. A lower concentration (5 mg NH_4–N · L^{-1}) than that recommended for optimum growth under conditions of continuous supply (Ingestad 1979) was chosen to simulate a low nutrient supply and to obtain a positive growth response on mycorrhizal infection. The amount of nutrient solution was standardized to 65 mL/pot after 10 days, which was given twice a week during the experiment. The positions of pots were randomized each week.

The stock water extract was diluted with distilled water to 3.75, 2.50, 1.50, 0.50, and 0% and used to water the seedlings. These concentrations were chosen because laboratory-made water extracts between 1.25 and 2.50% correspond to concentrations, which are toxic to seed germination, in snowmelt water collected under dense *E. hermaphroditum* vegetation (Zackrisson and Nilsson 1992).

At day 63 (39 days after transplanting), and once a week thereafter, 60 mL of each of the five dosages was added to each of eight inoculated and eight uninoculated seedlings (Table 1).

All solutions were added in excess of the water holding capacity of the pots to renew the soil solution by displacement. Four harvests, each comprising eight inoculated and eight uninoculated seedling per treatment, were made at 60, 81, 112, and 144 days after inoculation (Table 1). In total, 320 seedlings were used in the experiment. No extract was added during the last 20 days of the experiment.

Studies of plant growth and root systems

The seedlings were carefully separated from the sand, rinsed in water, and divided into shoot and root. Root length was determined by Newman's method (1966) as modified by Tennant (1975). Simultaneously, the total number of root tips and the number of root tips from dichotomous branches were counted. Root tips from dichotomous branches were assumed to be ectomycorrhizae formed by *Paxillus involutus* (as will be shown and further discussed below, dichotomy was very restricted among uninoculated plants). The shoots and roots were oven-dried (70°C, 48 h) to determine dry weights. Nitrogen concentration was analysed in shoots plus roots from the treatments 0, 1.50, and 2.50% *E. hermaphroditum* extract using a CHN elemental analyzer. Nitrogen content was calculated by multiplying by dry weight.

The method of estimating uptake of N per nondichotomous (uninfected) root tip per day and per dichotomous (mycorrhizal) tip per day

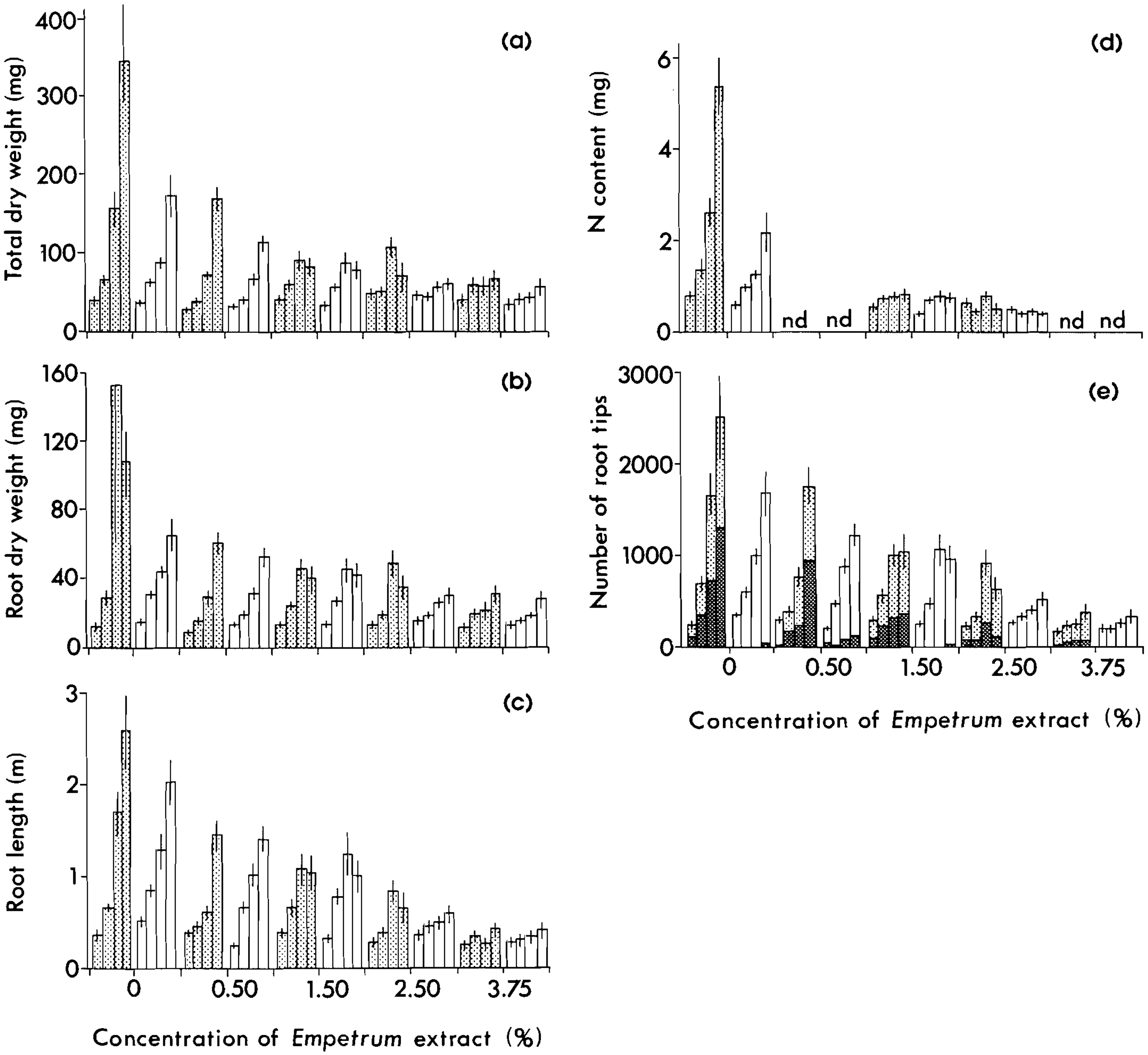

FIG. 1. Development of (*a*) total dry weight, (*b*) root dry weight, (*c*) root length, (*d*) total N content, and (*e*) number of root tips in seedlings of *Pinus silvestris* exposed to different concentrations of *E. hermaphroditum* extract. Quartets of bars represent sequential harvests from left to right. Shaded bars, inoculated (MYC) seedlings; open bars, uninoculated (NONMYC) seedlings. Dark shading indicates dichotomous root tips (*e*). Vertical bars indicate ± SE.

followed Högberg (1989), as described below.

Data on control seedlings at emergence and at the four harvests suggested an exponential increase in mass, number of root tips, and N content (see Results). Therefore, the average relative growth R_G between the harvests t_1 and t_4 was calculated for the different parameters as

$$[1] \quad R_G = \frac{\ln \text{mass}_{t_4} - \ln \text{mass}_{t_1}}{t_4 - t_1}$$

From this type of calculation exponential growth factors (R) were derived for the numbers of nondichotomous and dichotomous root tips. The increase in number of root tips from t_1 to t_4 could then be described by

$$[2] \quad f(t) = n_{t_1} \times \exp[R(t_4 - t_1)]$$

where n_{t_1} is the number of root tips at day t_1. Integration over the period between harvests gave the number of root-tip days that were responsible for the increase in N (ΔN) during that period. Thus, as uninoculated plants apparently remained nonmycorrhizal (see Results), the average N uptake rate per nondichotomous root tip (I_r) was calculated as

$$[3] \quad I_r = \frac{\Delta \text{N}}{\int_{t_1}^{t_4} n_{t_1} \times \exp[R(t - t_1)]\, dt}$$

It was further assumed that the N uptake rate by nondichotomous root tips was the same in inoculated as in uninoculated plants. This will be discussed further below. The total number of root-tip days (nondichotomous + dichotomous) minus the number of dichotomous root-tip days (both calculated by eq. 2) gave the number of nondichotomous root-tip days in mycorrhizal seedlings. This was multiplied by the uptake rate obtained in [3]. The remaining increase in N during the period between harvests was then divided by the number of dichotomous root-tip days to give the N uptake rate by dichotomous tips. Uptake by unit root length was calculated analogously, except that no distinction could be made between dichotomous and nondichotomous parts of the root systems. We also calculated uptake rate by a simple linear model using data from the first and last harvests.

Effects on the fungus Paxillus involutus *in pure culture*

Modified MMN medium (Marx 1969) was mixed with water extract of *E. hermaphroditum* (see above) to obtain 0.50, 0.25, 0.15, and 0% concentrations of extract of *E. hermaphroditum* in the medium. The mixture was autoclaved at 121 – 123°C for 40 min. The extract retained its toxicity after this heating (unpublished data). Twenty-five millilitres of medium was poured in each of 16 replicate 85-mm Petri dishes and inoculated with 7-mm diameter agar plugs cut from the edge of a *Paxillus involutus* stock fungus culture. Dishes were maintained in darkness at 20°C. Measurements of fungal colony diameters were made every 2nd day with a ruler for 26 days. As Petri dishes with contaminants were discarded, the final number of replicates ranged from 13 to 16.

Analysis of data

Three-factor analysis of variance was used to determine differences between uninoculated and inoculated plants, treatments and harvests in the main experiment. Unless shown otherwise, results are significant at the $p \leq 0.05$ level. Further details of the ANOVA are given in the Appendix.

Results

Effects on seedling development

Biomass

At the beginning of the experiment, inoculated (MYC) and uninoculated (NONMYC) Scots pine seedlings had similar biomass, but MYC seedlings rapidly became larger than NONMYC seedlings unless treated with *E. hermaphroditum* water extract (Fig. 1). With time, *E. hermaphroditum* water extracts significantly reduced shoot and root growth of both MYC and NONMYC seedlings; this effect was strongest on MYC seedlings. Seedlings (MYC and NONMYC) treated with the three highest *E. hermaphroditum* concentrations showed little growth over the experimental period. There was, however, no mortality among seedlings from any of the treatments.

Shoot to root ratio

MYC seedlings receiving no *E. hermaphroditum* extract had a larger shoot to root ratio than NONMYC seedlings (Fig. 2). At harvests 2 and 3 the ratio had decreased but thereafter increased in seedlings receiving no extract. Among extract-treated seedlings, the ratio decreased rapidly over the period of the experiment. In the three strongest extract treatments no difference in shoot to root ratio was found between MYC and NONMYC seedlings at the end of the experiment.

Root length

MYC and N-MYC seedlings receiving no *E. hermaphroditum* extract formed the longest root systems. The extract significantly inhibited length growth of roots (Fig. 1*c*). As with the effects on biomass, the inhibition increased with increasing concentrations of extracts. The decrease in root weight following extract treatments was more pronounced at low concentrations of extract than the effect on root length (Figs. 1*b*, 1*c*). Hence, roots of extract-treated plants appeared to be thinner.

Nitrogen content

Analysis of N content of total seedlings showed that MYC seedlings receiving no *E. hermaphroditum* extract absorbed

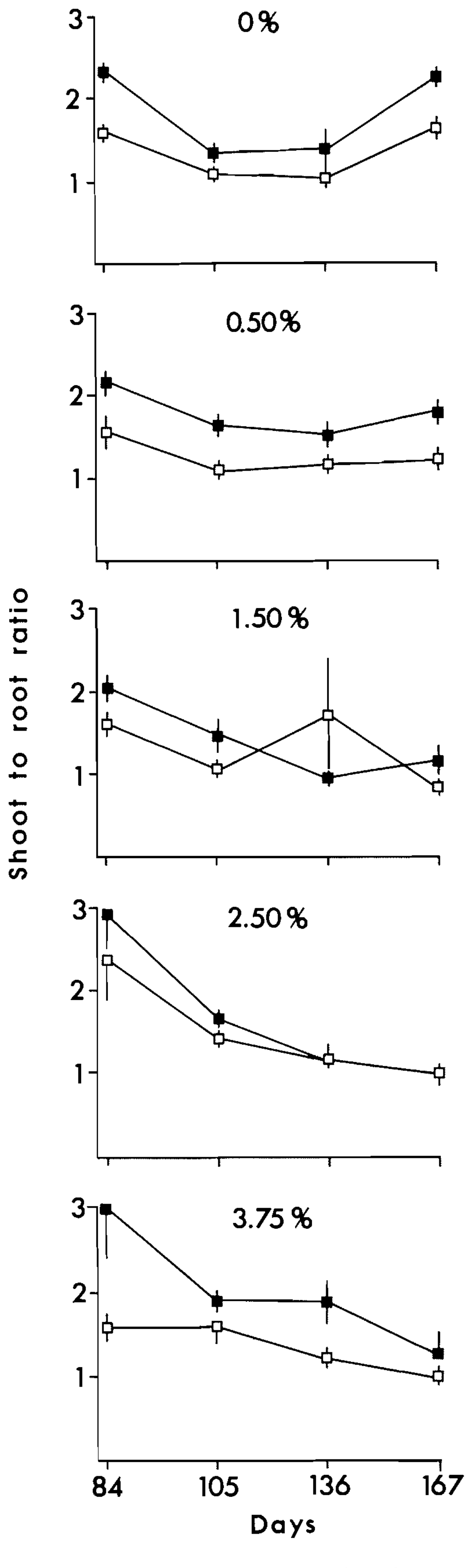

FIG. 2. Development of shoot to root ratios of *Pinus silvestris* seedlings exposed to different concentrations of *E. hermaphroditum* extract. ■, inoculated (MYC) seedlings; □, uninoculated (NONMYC) seedlings. Vertical bars indicate ± SE.

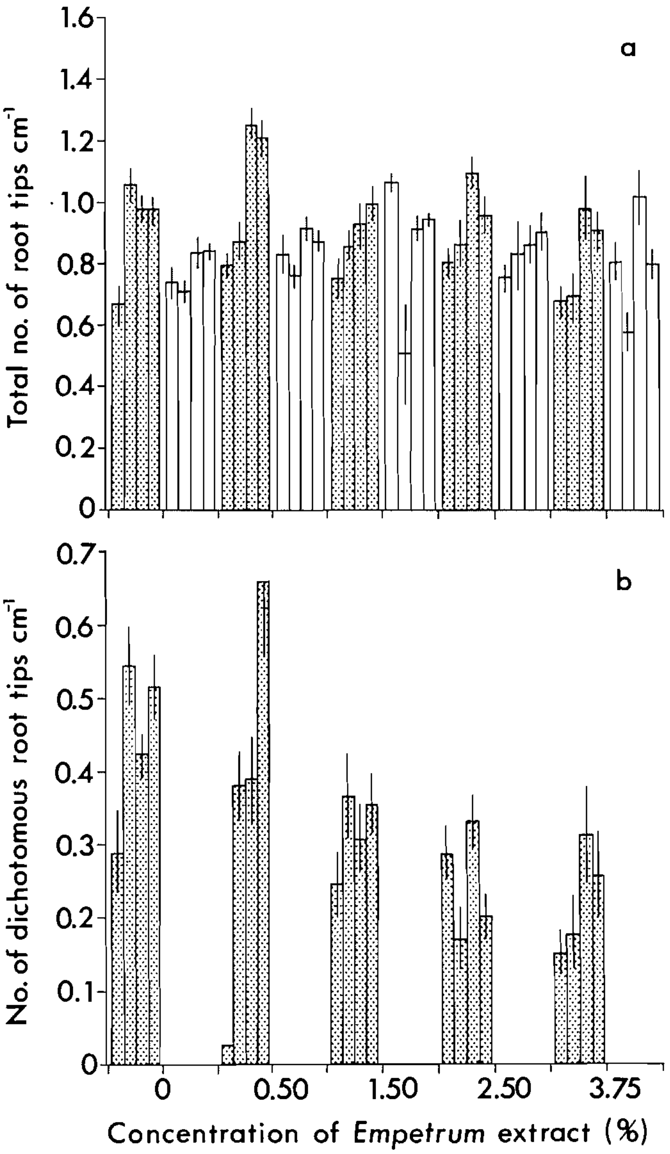

FIG. 3. (*a*) Total numbers of root tips per unit root length in *Pinus silvestris* seedlings exposed to different concentrations of *E. hermaphroditum* extracts. Shaded bars, inoculated (MYC) seedlings; open bars, uninoculated seedlings. Quartets of bars represent sequential harvests from left to right. (*b*) Numbers of dichotomous tips per unit root length. Vertical bars indicate ± SE.

TABLE 2. Concentrations of N (percentage of dry weight in shoot plus root) in *Pinus silvestris* seedlings at four harvests after additions of distilled water or two different concentrations of *Empetrum hermaphroditum* water extracts

Empetrum treatment (%)	Harvest (days)	Nitrogen concn. (%) MYC	Nitrogen concn. (%) NONMYC
0	84	1.9	1.6
0	105	2.0	1.6
0	136	1.6	1.4
0	168	1.6	1.4
1.5	84	1.4	1.2
1.5	105	1.2	1.2
1.5	136	0.9	0.8
1.5	168	1.0	1.0
2.5	84	1.2	1.1
2.5	105	0.8	1.0
2.5	136	0.7	0.8
2.5	168	0.7	0.7

NOTE: MYC, seedlings inoculated with *Paxillus involutus*; NONMYC, uninoculated seedlings.

TABLE 3. Three-way analysis of variance on the influence of mycorrhizal inoculation, treatment with extract of concentration of *Empetrum hermaphroditum*, and time of treatment on concentration of N (% dry wt.) in *Pinus silvestris* seedlings (shoot and root)

Parameter[a]	SS	*F*-ratio	df	*P*-value
MYC	0.5445	13.7	1	0.000
DOSE	18.9526	238.3	2	0.000
HARVEST	4.5643	38.3	3	0.000
MYC × DOSE	0.7039	8.8	2	0.000
MYC × HARVEST	0.0844	0.7	3	0.549
DOSE × HARVEST	0.5292	2.2	6	0.044
MYC × DOSE × HARVEST	0.1908	0.8	6	0.571
Error	6.6809		168	
Total	32.2506		191	

[a] MYC, MYC seedlings or NONMYC seedlings; DOSE, dosage of *E. hermaphroditum* water extract; *n* = 8.

more N than NONMYC seedlings and *E. hermaphroditum*-treated seedlings (Fig. 1*d*). The percentage of N in the plants declined with increasing dose of extract and with time regardless of mycorrhizal inoculation (Tables 2, 3).

Root tips and mycorrhizal infection

The NONMYC seedlings had formed few dichotomous roots, which implies that dichotomous branching in MYC seedlings was mainly due to mycorrhizal infection. The total number of root tips formed and percentage of dichotomous root tips were highest in MYC seedlings receiving no *E. hermaphroditum* extract (Fig. 1*e*). At the first harvest, 43% of the root tips among MYC seedlings were found to be dichotomous, a figure that increased to 53% by the end of the experiment. The formation of root tips among extract-treated MYC and NONMYC seedlings was slower and decreased with increasing concentrations of extract. The percentage of dichotomous branching among extract-treated MYC seedlings was much lower than in untreated MYC seedlings, except for the 0.50% concentration at the final harvest. At the two highest concentrations there was no significant increase in dichotomous root tips with time. There was no difference between treatments in the total number of root tips formed per unit root length (Fig. 3*a*). However, the number of dichotomous root tips formed per unit root length decreased with increasing concentration of the added extract (Fig. 3*b*).

Effects on nitrogen uptake

Uptake of N per root-tip day

Assuming an exponential increase in the number of root tips during the period from 84 to 168 days (Fig. 1*e*), the uptake of N into nondichotomous root tips of NONMYC seedlings receiving no *E. hermaphroditum* extract was calculated to be 22 ng N · day^{-1} per tip (Table 4). If nondichotomous tips took up N at the same rate in MYC seedlings as in NONMYC seedlings, the estimate of N uptake per tip per day was more than 4 times greater in dichotomous root tips than in nondichotomous tips (Table 4). Compared with untreated seedlings, NONMYC seedlings treated with 1.50% *E. hermaphroditum* extract took

TABLE 4. Uptake of N by nondichotomous and dichotomous root tips per day and per centimetre of root length per day during growth between 84 and 168 days of nonmycorrhizal (NONMYC) and mycorrhizal (MYC) *Pinus silvestris* seedlings watered with three different concentrations of *Empetrum hermaphroditum* water extract or distilled water

	0%		1.5%		2.5%	
	NONMYC	MYC	NONMYC	MYC	NONMYC	MYC
Nondichotomous roots (ng N · day^{-1} per root tip)	21.6 (18.6)	21.6 (18.6)[a]	7.5 (6.7)	7.5 (6.7)[a]	0	0 (0)[a]
Dichotomous roots (ng N · day^{-1} per root tip)	—	92.1 (59.3)	—	0.9 (1.6)	—	0 (0)
Total root system (ng N/(cm · day))	16.8 (14.7)	47.8 (37.2)	6.7 (6.2)	4.6 (4.3)	0 (0)	0 (0)

NOTE: Calculations are based on the assumption that growth of the various parameters was exponential. Results obtained using a simple linear model are shown in parentheses.

[a] Based on the assumption that nondichotomous tips take up N at the same rate in MYC seedlings as in NONMYC seedlings.

up 3 times less N by nondichotomous tips. On the basis of these assumptions, it was calculated that the N uptake by dichotomous tips of MYC plants treated with 1.50% *E. hermaphroditum* extract was 100 times less than in untreated MYC seedlings. Uptake of N by both nondichotomous and dichotomous root tips of MYC and NONMYC seedlings treated with 2.50% *E. hermaphroditum* extract was zero, because there was no increase of seedling N content between harvests 1 and 4. When a linear model of calculation was used, the same pattern was found, but the differences were somewhat smaller (Table 4).

Uptake of N per unit root length per day

Calculations of N uptake per centimetre of root length (total root system) revealed that MYC seedlings receiving no *E. hermaphroditum* extract were 3 times more effective in uptake than NONMYC seedlings (Table 4). When MYC seedlings were treated with extract at a concentration of 1.50%, the root system became 10 times less effective than untreated MYC seedlings, whereas NONMYC seedlings reduced their uptake per centimetre of root length by 2.5 times. Calculations using a linear equation resulted in sligthly smaller differences between treatments.

Effects on the fungus

Radial growth of *Paxillus involutus* was strongly reduced on the medium containing extract of *E. hermaphroditum*; the degree of reduction increased with increasing concentration of extract (Fig. 4). Even the most dilute concentration (0.15%) significantly reduced the diameter of growth of the fungus after only 2 days.

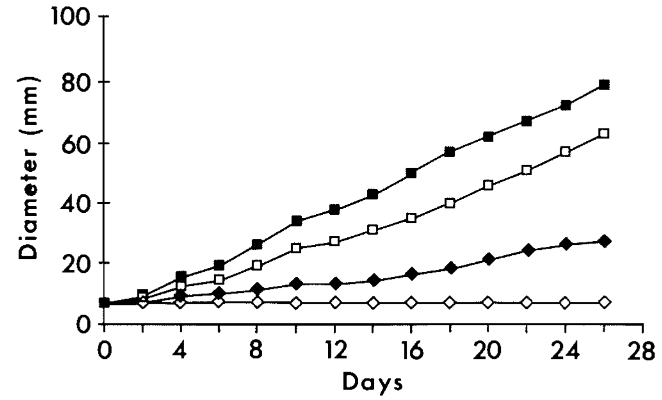

FIG. 4. Diameter growth of *Paxillus involutus* grown on MMN medium treated with *E. hermaphroditum* extract at four concentrations: ■, 0%; □, 0.15%; ◆, 0.25%; ◇, 0.50%.

Discussion

This study demonstrated that dry weight, root length, formation of root tips, and mycorrhizal infection of Scots pine were strongly reduced by substances in water extracts of *E. hermaphroditum* leaves. These parameters responded clearly when water extracts were added to the substrate of 63-day-old pine seedlings (Fig. 1). Nitrogen content of the plants was greatly reduced by *E. hermaphroditum*, which demonstrated an important inhibition of N uptake. The impairment of root function was further corroborated by the data on shoot to root ratios (Fig. 2) and concentrations of N (Table 2), i.e., carbon assimilation was less directly affected, which was indicated by the fact that N was diluted by growth.

We classified dichotomous roots as mycorrhizae, although we were aware that such roots may also form in the absence of mycorrhizal fungi (e.g., Faye et al. 1981). We used this criterion because we knew that very few dichotomous roots form on NONMYC Scots pine under these experimental conditions and because we lacked a better criterion that could be applied when counting very large numbers of root tips (in all ca. 250 000). Sectioning to confirm the presence of sheaths and Hartig nets is theoretically possible, but we decided not to undertake such a massive effort.

The rate of increase in total number of root tips was slower when higher concentrations of extracts were added (Fig. 1). This was due to smaller root systems, because there was no difference in total number of root tips formed per centimetre of root length between treatments (Fig. 3). However, the formation of dichotomous roots per centimetre of root length decreased with increasing dosages of *E. hermaphroditum* extract, and this effect was already apparent at the first harvest. Thus, *E. hermaphroditum* had a less marked effect on total length of roots and total number of root tips than on the number of dichotomous root tips.

The negative effects of extracts of *E. hermaphroditum* on the uptake of N, particularly on uptake of N by MYC plants, were evident irrespective of the method of calculating N uptake (Table 4). One may raise doubts about the classification of mycorrhizae, but the independent data on uptake per unit root length give strong support to our conclusion that the extract effectively removed the beneficial effect of mycorrhizal inoculation. Apparently there were multiplicative effects caused by the extract; root development (numbers of tips and length) as well as uptake per root tip or length decreased with increasing concentration of extract. Of these parameters N uptake per tip or length was clearly more sensitive than root growth alone (Table 4; Fig. 1), e.g., there was still some root growth, although uptake of N ceased when seedlings were treated with extract of the 2.50% concentration.

Very low concentrations of the extract restricted radial growth of *Paxillus involutus* in pure culture (Fig. 4). This suggests that the sensitivity of the fungus to *E. hermaphroditum* could have caused the slower mycorrhizal infection in roots of treated seedlings. Among fungal species tested by Coté and Thibault (1988), *Paxillus involutus* was shown to be the most sensitive to raspberry foliage extract. Similarly, Brown and Mikola (1974) indicated that it was the most sensitive of a range of species to *Cladonia alpestris* extract. Apart from the lower level of dichotomous branching, the low N content of MYC seedlings treated with *E. hermaphroditum* was apparently due to its effect in reducing the ability of mycorrhizae to take up N (Table 4). In fact, using the N-uptake rates by NON-MYC seedlings for nondichotomous tips in MYC seedlings may lead to an underestimate of the difference between dichotomous and nondichotomous tips (Högberg 1989), because a compensatory response in the plant can promote a greater uptake per unit root in loci (microsites), which it experiences as richer than the rest of the soil (Drew and Saker 1975).

Root cell elongation and cell division in radicles was stressed as particularly sensitive to allelochemicals (Muller 1965), but our results show that N uptake is even more sensitive. To our knowledge, this is the first study clearly demonstrating a negative effect of allelopathic interference on the function of mycorrhizae. In previous studies no quantitative data were obtained, e.g., Fisher (1979) showed that the morphology of the root system of jack pine (*Pinus banksiana* Lamb.) and white spruce (*Picea glauca* (Moench) Voss) was altered in the presence of lichens, but his data did not allow a distinction between effects on uptake by roots and effects on translocation of ^{32}P from roots to shoots.

Our earlier research showed that *E. hermaphroditum* significantly inhibits seed germination of Scots pine under natural conditions (Zackrisson and Nilsson 1992; Nilsson and Zackrisson 1992). Similarly, Scots pine grown in *E. hermaphroditum* vegetation grows more slowly compared with Scots pine grown in vegetation detoxified with activated carbon (Nilsson and Zackrisson 1992). In a greenhouse experiment, seed germination and radicle growth of aspen were significantly inhibited when exposed to throughfall passed over intact living *E. hermaphroditum* (Zackrisson and Nilsson 1992). Radicle elongation was inhibited (65% inhibition) more than seed germination (15% inhibition). Natural toxin dosage, if compared with the degree of inhibition caused by laboratory-made water extracts, may reach a concentration between 1.25 and 2.50%. However, the concentration of biologically active toxins may change due to adsorption to soil particles and breakdown by soil microorganisms (Rice 1984). We have shown, however, that substances leached from *E. hermaphroditum* in the field accumulate in the soil at levels affecting seed germination at the start of the growing season (Nilsson and Zackrisson 1992). Based on evidence obtained here, we argue that *Paxillus involutus* may be even more sensitive than seed germination and root growth of Scots pine when exposed to comparable concentrations of *E. hermaphroditum* extract (Zackrisson and Nilsson 1992). Although *Paxillus involutus* is not uncommon in our forests, we have no knowledge about its occurrence and distribution in forest soils dominated by *E. hermaphroditum*, or about its importance to regeneration of Scots pine. Although allelochemicals from *E. hermaphroditum* have considerable potential to retard root growth and reduce root function, the effect of these substances on other species of ectomycorrhizae and the potential effects under more complex situations in the field will require further study.

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Appendix

Three-way analysis of variance on shoot, root, and total dry weight, shoot to root ratio, root length, nitrogen content, total number of root tips, nondichotomous and dichotomous root tips, and number of root tips per root length in uninoculated *Pinus silvestris* seedlings and *Pinus silvestris* seedlings inoculated with *Paxillus involutus*

Parameter[a]	SS	*F*-ratio	df	*P*-value
Shoot weight				
MYC	0.0178	17.6	1	0.000
DOSE	0.0813	20.0	4	0.000
HARVEST	0.1094	35.9	3	0.000
MYC × DOSE	0.0238	5.9	4	0.000
MYC × HARVEST	0.0158	5.2	3	0.002
DOSE × HARVEST	0.1708	14.0	12	0.000
MYC × DOSE × HARVEST	0.0421	3.5	12	0.000
Error	0.2843		280	
Total	0.7455		319	
Root weight				
MYC	0.0062	3.3	1	0.069
DOSE	0.0513	6.9	4	0.000
HARVEST	0.0788	14.2	3	0.000
MYC × DOSE	0.0162	2.2	4	0.070
MYC × HARVEST	0.0106	1.9	3	0.126
DOSE × HARVEST	0.0444	2.0	12	0.024
MYC × DOSE × HARVEST	0.0240	1.1	12	0.376
Error	0.5171		280	
Total	0.7486		319	
Total weight				
MYC	0.0382	29.4	1	0.000
DOSE	0.1942	37.4	4	0.000
HARVEST	0.3295	84.6	3	0.000
MYC × DOSE	0.0322	6.2	4	0.000
MYC × HARVEST	0.0302	7.8	3	0.000
DOSE × HARVEST	0.3000	19.2	12	0.000
MYC × DOSE × HARVEST	0.0626	4.0	12	0.000
Error	0.3637		280	
Total	1.3507		319	
Shoot to root ratio				
MYC	12.8	31.1	1	0.000
DOSE	3.3	2.0	4	0.093
HARVEST	36.0	29.2	3	0.000
MYC × DOSE	3.5	2.1	4	0.079
MYC × HARVEST	4.0	3.2	3	0.022
DOSE × HARVEST	18.7	3.8	12	0.000
MYC × DOSE × HARVEST	5.1	1.0	12	0.411
Error	115.0		280	
Total	198.5		319	
Root length				
MYC	107	0.1	1	0.789
DOSE	309234	51.8	4	0.000
HARVEST	312478	69.8	3	0.000
MYC × DOSE	6759	1.1	4	0.341
MYC × HARVEST	6254	1.4	3	0.244
DOSE × HARVEST	204671	11.4	12	0.000
MYC × DOSE × HARVEST	25190	1.4	12	0.162
Error	417563		280	
Total	1282256		319	
Nitrogen content				
MYC	11.74	34.2	1	0.000
DOSE	69.23	100.8	2	0.000
HARVEST	33.92	32.9	3	0.000
MYC × DOSE	13.84	20.2	2	0.000
MYC × HARVEST	7.83	7.6	3	0.000
DOSE × HARVEST	55.94	27.2	6	0.000
MYC × DOSE × harvest	15.60	7.6	6	0.000
Error	57.00		166	
Total	263.99		189	
No. nondichotomous root tips				
MYC	1421645	21.3	1	0.000
DOSE	10838542	40.6	4	0.000
HARVEST	17425108	87.1	3	0.000
MYC × DOSE	681245	2.6	4	0.039
MYC × HARVEST	257799	1.3	3	0.279
DOSE × HARVEST	6872342	8.6	12	0.000
MYC × DOSE × HARVEST	1155406	1.4	12	0.146
Error	18680720		280	
Total	57332804		319	
No. dichotomous root tips				
MYC	5624362	182.8	1	0.000
DOSE	3403260	27.6	4	0.000
HARVEST	3282491	35.5	3	0.000
MYC × DOSE	2712085	22.0	4	0.000
MYC × HARVEST	2621626	28.4	3	0.000
DOSE × HARVEST	3116194	8.4	12	0.000
MYC × DOSE × HARVEST	2616820	7.1	12	0.000
Error	8617217		280	
Total	31994056		319	

Appendix (*concluded*)

Parameter[a]	SS	*F*-ratio	df	*P*-value
Total no. root tips				
MYC	1390622	10.5	1	0.001
DOSE	25598624	48.2	4	0.001
HARVEST	35270596	88.5	3	0.000
MYC × DOSE	1279839	2.4	4	0.000
MYC × HARVEST	1385160	3.5	3	0.050
DOSE × HARVEST	18078724	11.3	12	0.016
MYC × DOSE × HARVEST	2953168	1.8	12	0.000
Error	37189580		280	0.040
Total	123146312		319	
Total no. root tips per root length				
MYC	87.7	17.3	1	0.000
DOSE	77.3	3.8	4	0.005
HARVEST	255.3	16.8	3	0.000
MYC × DOSE	26.2	1.3	4	0.274
MYC × HARVEST	107.3	7.1	3	0.000
DOSE × HARVEST	94.3	1.6	12	0.106
MYC × DOSE × HARVEST	79.2	1.3	12	0.217
Error	1418.6		280	
Total	2145.9		319	
No. dichtomous root tips per root length				
MYC	652.0	571.5	1	0.000
DOSE	85.8	18.8	4	0.000
HARVEST	35.6	10.4	3	0.000
MYC × DOSE	35.0	7.7	4	0.000
MYC × HARVEST	67.4	19.7	3	0.000
DOSE × HARVEST	51.4	3.8	12	0.000
MYC × DOSE × HARVEST	109.4	8.0	12	0.000
Error	319.4		280	
Total	1356.0		319	

NOTE: The seedlings were treated with distilled water (control) or different concentrations of water extract of *E. hermaphroditum* and harvested four times between 84 and 168 days of treatment.

[a] MYC, MYC or NONMYC seedlings; DOSE, dosage of *E. hermaphroditum* water extract or distilled water; *n* = 8.